REMARKS

Applicant respectfully requests reconsideration. Claims 30-41, 70-74 and 80-83 were previously pending in this application. No new matter has been added.

Rejection Under 35 U.S.C. § 103

The Examiner rejected claims 30-40, 70-74 and 80-83 under 35 U.S.C. 103(a) as unpatentable over Fire et al. (WO 99/32619 A1, the "Fire PCT application") in view of Noren et al. (U.S. 5,691,140), Conkling et al. (U.S. 5,459,252) and Talkad et al. (J. Bacteriol. 135: 528-541, 1978). Applicant respectfully traverses the rejection and requests reconsideration.

The instant claims are related to micro-organisms comprising an expression vector with promoters flanking a DNA sequence for production of double stranded RNA in said micro-organism. Applicant submits that these claimed bacterial vectors and expression systems for dsRNA would be of no use in the process of Fire et al., i.e., inhibiting endogenous genes in cancer cells.

The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the expression vector construct of Fire et al. by incorporating the teaching of Noren et al. (of a vector with two identical promoters) and Conkling et al. (root specific promoters) so as to produce an organism comprising an expression vector that allows desired transcription and expression of the double stranded RNA within the organism, and that one of ordinary skill in the art would be motivated to do so.

Applicant respectfully disagrees with the rejection for the following reasons, and provides herewith a description of the teachings of the prior art references combined by the Examiner in order to clarify the differences between the combination of prior art and the claimed invention.

Fire et al.

The Fire PCT application is alleged by the Examiner to teach a micro-organism comprising an expression vector that produces double stranded RNA. The Examiner asserts on page 4 of the Office Action that the Fire PCT application teaches "that the micro-organism comprising the expression vector can be any organism including plant, animal, protozoan, virus, *E. coli*, yeast, and parasitic nematode" and that "all of the teachings are also disclosed in their priority documents U.S. 60/068,562". Applicant strongly disagrees that U.S. 60/068,562 supports the asserted subject matter of the Fire PCT application.

The Examiner states that "Fire et al. teach *C. elegans* comprising an expression vector having an RNA polymerase promoter selected from T7, T3 and SP6 thereby producing double stranded RNA in *C. elegans in vivo*." (Office Action at pages 3-4.) Applicant does not find a single passage in the Fire priority application describing that RNA is produced "inside" *C. elegans* by a micro-organism *in vivo*. All double stranded RNA is introduced by injection.

According to the Examiner, Fire does not teach an expression vector comprising two promoters wherein the promoters are root-specific promoters. The Examiner combines the Fire PCT application with the teachings of Noren et al. (bidirectional expression vectors) and Conkling et al. (root specific promoters).

Applicant submits that U.S. application 60/068562 (the "Fire provisional") does not disclose the subject matter asserted by the Examiner. Accordingly, the effective filing date of the Fire PCT application for this subject matter does not extend to the filing date of the Fire provisional, for at least the following reasons. The Fire provisional does not disclose elements of the claimed invention, i.e., a micro-organism comprising an expression vector that comprises promoters flanking a DNA sequence such that the promoters initiate transcription of said DNA sequence to produce double stranded RNA, nor any use of such a micro-organism. Applicant explained, in responses to previous Office Actions, the deficiencies of the Fire provisional and differences in disclosure between the Fire provisional and the Fire PCT application. Some of these deficiencies and differences are again explained below.

The Fire provisional application discloses that double stranded RNA can be synthesized *in vitro* or *in vivo* (see, e.g., page 7, lines 11-15; page 11, lines 17-24; page 15, line 5), but does not disclose that the double stranded RNA can be produced *in vivo* in a micro-organism. The Fire provisional discloses that double stranded RNA that is produced is introduced into cells or organisms with the target gene (i.e., the organism in which the RNA interference effect is desired) primarily by injection (see throughout the application, e.g., at page 7, lines 16-19; figure legends to Figs. 2-4 at pages 8-10; page 12, lines 1-12; page 16, lines 18-23; Table 1). The Fire provisional discloses that strands can be *purified and annealed prior to injection* (see, e.g., page 11, lines 23-29; page 15, lines 16-26, page 17, lines 23-26).

There is no description in the Fire provisional, however, of introducing replicating nucleic acids (and, more particularly, micro-organisms producing the nucleic acids) in cells or organisms with the target gene (see page 5 lines 18-21, especially last part of the sentence). The RNA or double stranded RNA is mainly introduced by injection in the cells or organisms with the target gene (see page 7, lines 16-19; pages 8-10: description of figures; page 12 lines 1 to 10); and only in one paragraph, one method is taught for introducing it into cells with the target gene (e.g., cancer cells), namely by viral vectors (page 12, lines 10-12). Again no mention is made of bacterial vectors.

The instant specification describes a micro-organism as, for example "a bacterial or yeast cell, which may be fed to the organism", which "may be adapted to express the appropriate transcription factor" and in a preferred embodiment, that "the microorganism is *E. coli*." Page 2, lines 27-30; see also the working examples and claims 39-41 as originally filed. A person or ordinary skill in the art would not, however, consider a virus, a plant, an animal, or a nematode (including *C. elegans*) to be a micro-organism. With respect to a virus, it cannot be a micro-organism as claimed because it does not meet the limitation of claim 30 that "double stranded RNA is produced in said micro-organism", since while the RNA could possible be encoded by a viral nucleic acid, viruses are not capable of producing RNA other than by using proteins produced by host cells.

Clearly, the Fire provisional does not disclose producing double-stranded RNA from an expression vector *in vivo* in a micro-organism, and therefore does not provide priority for the aspects of the cited Fire PCT application that relate to Applicant's claimed invention.

Noren et al.

The vectors described in Noren et al. are for *in vitro* transcription of either strand of DNA, i.e. for the production of single stranded DNA. It appears that the Examiner recognized this in stating on page 4 of the Office Action that Noren et al. teach "bidirectional expression vectors comprising two promoters in opposite direction allow[ing] efficient transcription of either strand of the inserted piece of RNA sequence." (emphasis added) The intended use of these vectors is the production of RNA only when *one of the promoters has been rendered inoperable*, such as by cleavage with a restriction endonuclease (see the Noren et al. patent throughout, e.g., the claims). For example, as noted in col. 3, lines 49-52, "The direction of transcription is thus determined by which enzyme is used to linearize DNA template prior to transcription…." There is nothing in the Noren et al. patent that teaches or suggests using these vectors for transcription of *both sense and antisense strand at the same time* for production of double stranded RNA.

Conkling et al.

The Examiner states that Conkling et al. teach that root-specific promoter RB7 can be used in an expression vector to permit root-specific transcription and expression of an exogenous nucleotide sequence in a plant cell. Office Action at page 4. Applicant submits that Conkling et al. teaches nothing additional that is relevant to the instant claimed invention, and therefore does not make up for any deficiencies in the other references combined by the Examiner.

Talkad et al.

According to the Examiner, the Talkad et al. reference describes *E. coli* strains deficient in RNase III and that RNase III cleaves bacteriophage T7 RNAs as well as double-stranded

RNAs. According to the Examiner, it would have been obvious to substitute the *E. coli* strains deficient in RNase III of Talkad et al. for the strains used in the Fire PCT application.

Applicant's response is that even if such a substitution was made, the Talkad et al. reference does not supply any other of the elements of the claimed invention that are missing from the Fire PCT application, the Noren patent and the Conkling patent.

The combination of prior art references does not teach or suggest the invention

There was no recognition in the combination of cited prior art references that expression vectors could be used for production of dsRNA, not *in vitro*, but in a micro-organism, e.g., a bacterial cell. In particular, the combination of references does not teach or suggest a micro-organism having an expression vector that comprises promoters flanking a DNA sequence such that the promoters initiate transcription of said DNA sequence to produce double stranded RNA in the micro-organism upon binding of a transcription factor to said promoters,

Nor was there any need, reason or motivation to make the invention as claimed. At the time that the invention was made, expression vectors were used to produce single stranded RNA *in vitro* (e.g., for RNA probes); this was a method commonly used to produce *single-stranded* RNA. As of its priority date, Fire et al. did not disclose or motivate the person of ordinary skill in the art to produce *double stranded* RNA in a micro-organism, e.g., a bacterial system, since Fire et al. produced dsRNA by synthesizing single strands of RNA and then annealing the single strands to produce dsRNA. Indeed, the primary use of dsRNA in the Fire provisional was to inhibit endogenous target genes in cancer cells for which a micro-organism (e.g., bacterial) expression system was not taught or suggested and in fact is of no use.

Therefore, there was no reason or motivation for one of ordinary skill in the art at the time the invention was made to modify the expression vector constructs of Fire et al. (as described in the Fire provisional, for producing single stranded RNA), Noren et al. (vectors for *in vitro* transcription of single stranded RNA) and Conkling et al. (root specific promoters) so as to produce a micro-organism comprising an expression vector that allows desired transcription and

expression of the double stranded RNA within the micro-organism. In particular, the skilled person certainly would not have had a reason to combine or have been motivated to combine the teachings of these references to make or use a microorganism that produces double stranded RNA as claimed by Applicant because none of the references teaches or suggests production of double stranded RNA in a micro-organism. It is only due to the inventive contribution of Applicant that the claimed invention was produced.

In summary, the combination of references does not provide all of the elements of the claimed invention, and a sufficient reason or motivation to combine the references has not been provided as is required for a finding of obviousness. Thus the combination of references does not render the claimed invention obvious. Accordingly, Applicant respectfully requests withdrawal of the rejection of claims 30-40, 70-74 and 80-83 under 35 U.S.C. § 103(a).

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
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Docket No.: D0590.70011US02
Date: January 30, 2009
X02/01/09x